Rhodobacter capsulatus puc operon: promoter location, transcript sizes and effects of deletions on photosynthetic growth

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Gene deletions of the puc operon of Rhodobacter capsulatus showed that the pucC and pucE genes, but not pucD, were required for formation of wild-type levels of the LHII complex. Deletion of pucC or pucE also impaired photosynthetic growth. The effects of pucC deletion were suppressed by secondary mutations that mapped outside the puc operon. Fusion of alacZgene to pucE showed that most of pucE transcription originated from upstream of pucE. RNA blot analysis revealed a 2.4 kb transcript that hybridized to probes specific for the pucBA, pucC and pucDE regions, indicating that some puc operon messages extend from just before the pucB gene to just after the pucE gene.

Introduction

The integral membrane protein components of the photosynthetic apparatus of the bacterium Rhodobacter capsulatus include the reaction centre complex (the site of charge separation), and the two surrounding light harvesting complexes, light harvesting I (LHI; sometimes designated B870) and light harvesting II (LHII; B800–850) (reviewed by Kiley, 1985). The two pigment-binding peptides of the LHII complex are encoded by the pucBA genes (Youvan & Ismail, 1985). Two dicistronic RNA species of approximately 550 nt in length encoding these genes have been characterized (Zucconi & Beatty, 1988), and the puc operon was thought to consist of only these two genes. The LHII mutant strain NK3 was then discovered to have a transposon inserted downstream of the pucBA genes, and three new open reading frames (ORFs) were discovered in this region by DNA sequencing (Tichy et al., 1989). The new ORFs were named the pucC, pucD and pucE genes, since a transposon disruption of the pucC gene led to loss of the LHII complex, and the predicted protein sequence of the pucE gene matched the partial amino acid sequence of one of the two 14 kDa proteins that comodeled with the LHII complex (Tichy et al., 1989). The organization of these genes is given in Fig. 1(a).

Because the transposon insertion mutation of the pucC gene in strain NK3 (Tichy et al., 1989, 1991) could be polar on the pucD and pucE genes, and interfere with their expression, it was not known whether one or more of the pucC, pucD and pucE genes were required for formation of the LHII complex. Furthermore, although the pucC and pucE genes were reported to be required for normal levels of LHII complex (Tichy et al., 1991), the effects of puc gene deletions on growth has not been reported. It was also not known if all of the pucCDE genes were transcribed from the pucBA promoter region, or whether there was a promoter located between the pucC and pucDE regions. However, a complementation analysis indicated that the pucC gene could not be expressed if the pucBA promoter region was not present (Tichy et al., 1989).

For these reasons, we investigated the requirements of the pucC, pucD and pucE genes, individually, for cell growth and formation of the LHII complex. We created a mutant strain in which the chromosomal pucCDE genes were replaced by an omega cartridge (Prentki & Krisch, 1984), and complemented this deletion with plasmids carrying various combinations of puc operon genes. Plasmid-borne pucE::lacZ gene fusions were used to evaluate the location of the promoter(s) of the pucCDE genes. RNA species that hybridized to probes specific for the pucBA, pucC and pucDE regions, and were sufficiently long to encode the entire pucBADE region, were detected in wild-type cells.
puc operon deletion strain, R. capsulatus ALHII, was used for analyses. Capacities and shaking at 150 r.p.m. Stationary-phase low-oxygen conditions were obtained by filling flasks to 80% of their nominal concentrations shaken at 300 r.p.m. in an orbital shaker. Low aeration growth supplemented with the appropriate antibiotics at the following concentrations: ampicillin, 200 μg ml⁻¹ and spectinomycin sulphate, 100 μg ml⁻¹.

Methods

Bacterial strains and growth conditions. All subcloning was done in the Escherichia coli strains JM83 (Yanisch-Perron et al., 1985), an hsdR derivative of C600 (Bibb & Cohen, 1982), RB404 (Brent & Ptashne, 1980) and SM10 (Simon et al., 1983). Strain SM10 was used to transfer plasmids by conjugation to R. capsulatus (Simon et al., 1983). E. coli strains were grown at 30 °C in Luria broth (Maniatis et al., 1982) and puc operon deletion/insertion strain ALHII.

Construction of R. capsulatus strain ΔLHII. The 4.5 kb PstI-EcoRI fragment bearing the pucBACDE genes (Fig. 1a) from pRPSLHII (Youvan & Ismail, 1985) was first subcloned into pUC13 (Yanisch-Perron et al., 1985) for ease of subsequent manipulations, yielding pHLB1. After digestion of pHLB1 with Clal and partial digestion with BclI, the 4.6 kb vector fragment containing the sequences 3' of the Clal site at position 3030 of the published sequence (Tichy et al., 1989) and 5' of the Clal site in the puc operon (including the first 24 codons of the pucB gene) was purified, and treated with the Klenow fragment of DNA polymerase 1 to generate blunt ends (Maniatis et al., 1982). This fragment was recircularized, inserting a BamHI linker (5'-CCGGAT-CGG-3') at the ligation site by linker tailing (LaThe et al., 1984). The omega fragment (Prentki & Krisch, 1984) was then inserted into this BamHI site. The resultant ApucBACDE::Ω PstI-EcoRI fragment was inserted into the suicide vector pSUP202 (Simon et al., 1983) and introduced into SB1003 by conjugation. Spectinomycin resistant colonies were selected, and lighter coloured colonies were screened for loss of LHII by spectral analysis. Replacement of the puc operon as a result of a double cross-over event was confirmed by Southern blot analysis, as shown in Fig. 1 and described below.

DNA from SB1003 and ΔLHII (5 μg) was separately doubly digested with EcoRI and BamHI, run on a 1% (w/v) agarose gel and transferred to nitrocellulose paper (Maniatis et al., 1983). A non-radioactive DNA labelling kit (Boehringer Mannheim) was used to prepare digoxigenin-labelled probes from gel-purified DNA fragments. The blots were treated according to the kit's specifications until the development stage. When a fluorescent dye substrate for alkaline phosphatase (Gibco-BRL) was used to develop the blots, after which they were exposed to X-ray film (Kodak X-OMat) at room temperature for varying lengths of time before development.

As can be seen in Fig 1c, a single band, corresponding to the 5.5 kb EcoRI fragment that hybridized to the puc operon probe, was obtained with SB1003 DNA. In contrast, ΔLHII DNA yielded two smaller bands, approximately 2.3 and 0.9 kb in size, which we interpret to represent fragments that consisted of the puc operon flanking sequences from the ends of the omega cartridge (delimited by the BamHI sites) to the EcoRI sites.

Complementation plasmid constructions. The following deletions were made in the plasmid pHLB1 (see above) and transferred to the broad host-range plasmid pRK415 (Keen et al., 1988) as PstI-EcoRI fragments (Fig. 2). The omega fragment was inserted at the HindIII site upstream of the PstI site to reduce transcriptional read-through from plasmid promoters (Wellington et al., 1991). The positions of restriction enzyme sites given below refer to the numbering in the published DNA sequence (Tichy et al., 1989).

The pucCDE genes were deleted from the BeII site at position 1463 to the BeII site at 3030, yielding plasmid pACDE. The deletion of the pucC gene extended from the BeII site at position 1463 to the BsuBI site at 2217, forming plasmid pAC. Fusion of the filled-in BeII site to the blunt BsuBI end created a translational stop codon.

A translationally in-frame deletion of pucD was created by filling in the BeII site at position 2348 and the BssHI site at 2598 followed by religation (pΔD). The pucE deletion extends from the Eco47HI site at 2761 to the BeII site at 3030 (pΔE). To ensure that translation beginning at the pucE start codon did not continue into the presumed transcription stop signal downstream of the deletion, an in-frame translational stop codon was created by adding a BamHI linker.
Results

Effects of deletion of chromosomal pucBACDE genes and complementation with segments of the puc operon on LHII complex absorption spectra

Strain ΔLHII (Fig. 1 b) was created by deletion of 2.5 kb from the ClaI site in the 25th codon of pucB to the BclI site in the termination codon of pucE, and replacement with the omega fragment (see Methods). Strain ΔLHII-(pRK415::Ω) lacked the LHII complex, as evidenced by the loss of absorption at 800 and 850 nm (Fig. 3 d). Wild-type levels of LHII could be restored by complementation in trans with the plasmid pBACDE, which contained the entire puc operon (Figs 2 and 3 a).

A series of plasmids was made that carried different deletions of the pucC, pucD and pucE genes (Fig. 2), and these were conjugated into the ΔLHII strain. Spectral analysis of cells grown under conditions of low aeration showed that strains ΔLHII(pΔC) and ΔLHII(pACDE) had undetectable levels of LHII absorption (Figs 3 f and 3 e, respectively). In contrast, strain ΔLHII(pΔE) showed about 64% of the level of LHII complex absorption (based on computer integration of the areas under the 800 nm peaks) found with ΔLHII(pBACDE) (Fig. 3 c), whereas the spectrum of ΔLHII(pΔD) showed no difference from the profile obtained with ΔLHII(pBACDE) (Fig. 3 b).
Growth characteristics of puc gene deletions and some properties of secondary mutant strains

Because the LHII complex is thought to increase the intracellular area of photon absorption and thus might aid photosynthetic growth at low light intensities (Drews, 1985), the puc gene deletion strains were tested for ability to grow photosynthetically at the low light intensity of 30 μmol m⁻² s⁻¹ (Fig. 4a). At this intensity of light, strain ΔLHII(pBACDE) was light-limited for growth, with a doubling time of 12 h, whereas at saturating light intensities the doubling time was typically about 2 h (see below).

Surprisingly, strain ΔLHII(pRK415::Ω) grew only slightly slower than ΔLHII(pBACDE), despite undetectable levels of the LHII complex (Fig. 3d). The kinetics and extent of growth of ΔLHII(pΔD) at 30 μmol m⁻² s⁻¹ were indistinguishable from ΔLHII-(pBACDE). Strain ΔLHII(pΔE), which had lower than normal levels of LHII (see Fig. 3c), had a much slower doubling time than strain ΔLHII(pBACDE) and did not reach as great a density in stationary phase. Strain ΔLHII(pΔE) was tested for emission of fluorescence (Zilsel et al., 1989) to evaluate whether the LHII complex present might function improperly, but the fluorescence detected was similar to that of ΔLHII(pBACDE) or ΔLHII(pΔD) (not shown).

The growth of strains ΔLHII(pΔCDE) and ΔLHII-(pΔC) was the most impaired of those tested (Fig. 4a). Occasionally, faster-growing secondary mutants arose, distinguished by a marked increase in the slope of the growth curve and, in the case of ΔLHII(pΔC), by an unusual greenish colour. Aerobically-incubated plates streaked from faster growing ΔLHII(pΔCDE) and ΔLHII(pΔC) photosynthetic cultures showed two colony types: pink colonies similar to the original ΔLHII-(pΔCDE) or ΔLHII(pΔC) strains, and darker red colonies. Spectra of pure cultures started from these dark pigmented colonies [designated ΔLHII-1(pΔC) and ΔLHII-2(pΔCDE)] showed that the cells had regained LHII complex absorption, but to different extents (Fig. 3e and f). Strain ΔLHII-2(pΔCDE) had approximately wild-type levels of LHII complexes whereas ΔLHII-1(pΔC) had much lower amounts of the LHII complex. These secondary mutant strains grew photosynthetically under light intensities of 30 μmol m⁻² s⁻¹ and 300 μmol m⁻² s⁻¹ at rates similar to that of strain ΔLHII-(pBACDE) (not shown). The appearance of secondary mutants was not observed in cultures of any of the other strains described here, or in ΔLHII(pΔC) or ΔLHII-
puc operon gene deletions in *R. capsulatus*

I I

Time (h) 100 200 300

I Y

Y

Y g 100 8

8 10 100 8 10 20 30 40 50 60 70 80

Time (h)

Fig. 4. Photosynthetic growth of cells with illumination at (a) 30 μmol m⁻² s⁻¹ and (b) 300 μmol m⁻² s⁻¹. Strains: ○, ΔLHII(pBACDE); □, ΔLHII(pRK415::Ω; △, ΔLHII(pACDE); ▽, ΔLHII(pAD); ●, ΔLHII(pAE). The growth of ΔLHII(pACDE) at low light intensity (a) shows the appearance of a secondary mutant at approx. 80 h. In experiments where none arose the growth curve was identical to that of ΔLHII(pAC) (not shown).

(pACDE) cultures grown aerobically or photosynthetically with a light intensity of 300 μmol m⁻² s⁻¹. Because the ΔLHII(pAC), ΔLHII(pACDE) and ΔLHII(pAE) strains grew more slowly than ΔLHII(pRK415::Ω) at a low light intensity, we grew the strains photosynthetically at 300 μmol m⁻² s⁻¹ to determine if they were also impaired in photosynthetic growth at high light intensity. Strains ΔLHII(pBACDE), ΔLHII(pAD) and ΔLHII(pRK415::Ω) grew equally well with a doubling time of about 2 h (Fig. 4b). Strain ΔLHII(pAE) grew more slowly with a doubling time of approx. 7 h, whereas strains ΔLHII(pAC) and ΔLHII(pACDE) grew very poorly, with about 10 h doubling times.

**pucE gene promoter location by deletion analysis of pucE′::lacZ gene fusions**

Although previous work had shown that the pucC gene is probably transcribed from the pucBA promoter (Tichy et al., 1989, 1991), it was not clear where transcription of the pucDE genes originates. Therefore, to test for the positions of other possible puc operon promoters, in-frame fusions of the pucE gene were made to the *E. coli lacZ* gene (Fig. 5) in the promoter-probe plasmid pXCA601 (Adams et al., 1989). The specific activities of β-galactosidase were measured in cell extracts of *R. capsulatus* strain SB1003 containing the different pucE′::lacZ fusions, grown under either high or low aeration (see Methods). Fig. 5 shows that approximately 90% of the expression of the pucE′::lacZ fusion under low aeration derives from transcription initiated upstream of pucB. The remaining 10% of β-galactosidase activity was lost when the region between the 3′ end of the pucC gene and the 5′ end of the pucD gene was deleted.

Comparison of β-galactosidase specific activities between SB1003(pPEZ) cells grown under high and low aeration shows that expression of this pucE′::lacZ fusion averaged 1.8-fold higher in cultures that were oxygen-starved. This is in good agreement with the
Fig. 6. RNA blot analysis of puc operon transcripts. (a) DNA probes used. Symbols and abbreviations as in Fig. 2. (b) Autoradiograms of hybridized blots of RNA isolated from SB1003 (lanes 1, 3–5) and ΔLHII (lane 2) cells grown under low aeration (see Methods). Lanes 1 and 2 were probed with the 2.2 kb ClaI BspHI fragment (probe 1), which contains the pucBACDE genes; lane 3 with the 435 nt BanII fragment (pucBA, probe 2); lane 4 with the 1.1 kb BanII fragment (pucC, probe 3); and lane 5 with the 635 nt BspHI fragment (pucDE, probe 4). Approximate positions of RNA size markers (kb) are indicated beside the blots.

results obtained using a pucB:iac’Z fusion in cells grown under the same conditions (Forrest et al., 1989).

RNA blot analysis of the puc region shows several RNA species

Because pucE::iac’Z fusions showed that most, if not all, of transcription of the pucE gene originated upstream of the pucB gene, RNA blot analyses were performed to determine whether RNA species long enough to encode all five of the puc genes could be detected in addition to the approx. 550 nt pucBA messages previously characterized (Zueconi & Beatty, 1988). When RNA from the wild-type strain SB1003 was probed with a 2.2 kb fragment extending from the ClaI site in pucB to the BspHI site in pucE (probe 1 in Fig. 6a), a 0.5 kb message was detected along with two other less abundant species (Fig. 6b, lane 1). These two weaker signals corresponded to sizes of 2.4 kb and 1.0 kb. (The faint bands of approx. 1.5 kb visible in lanes 1, 2 and 3 are artifacts caused by rRNA bands above and below this position that interfere with hybridization.) By scanning the autoradiogram on a densitometer, the relative amounts of the 0.5 kb:1.0 kb:2.4 kb species were estimated as 35:3:1.

The 2.4 kb species described above was large enough to encode all five puc genes. In order to evaluate whether
it might, and to identify the sequences present in the smaller species, further RNA blot analyses were carried out using probes specific for smaller segments of the puc region (Fig. 6a). A probe specific for the pucBA genes detected only the largest (2.4 kb) and smallest (0.5 kb) bands (Fig. 6b, lane 3). A probe specific for the pucD and pucE genes was expressed in the mutants NK3 or U72 (Tichy et al., 1991). In that report the possibility of a strong promoter located between the three genes downstream of pucBA are required to likely polar effects of the transposon insertion and the these previous experiments were complicated by the authors suggested that deletion of the entire puc region was lethal, whereas we found that deletion of the entire puc region detected with the pucBA-containing probes. Although some signals are over-exposed in Fig. 6, to better show the faint bands, autoradiograms of shorter exposures of these and other blots clearly showed the presence and absence of the bands noted above.

When RNA from the ΔLHII strain was probed with the pucBACDE fragment no bands were detected (Fig. 6b, lane 2), indicating that none of the signals resulted from cross-hybridization to non-puc operon derived messages, as was reported with Rhodobacter sphaeroides (Lee et al., 1989).

Discussion

Effects of deletion of chromosomal pucBACDE genes and complementation with segments of the puc operon on LHII complex absorption spectra

This report describes the deletion of the entire puc- BACDE region and demonstrates that all of the genes downstream of pucBA are transcribed primarily from a promoter upstream of pucB. Previous studies of the expression of the pucD and pucE genes in R. capsulatus drew conclusions from studies of a mutant with a transposon inserted in the pucC gene (strain NK3), or a deletion of only the pucB, pucA and part of the puc operon genes (strain U72) (Tichy et al., 1989, 1991). The interpretations of these previous experiments were complicated by the likely polar effects of the transposon insertion and the possibility of a strong promoter located between the pucC and pucD genes. Therefore, it is not clear whether the pucD and pucE genes were expressed in the mutants NK3 or U72 (Tichy et al., 1991). In that report the authors suggested that deletion of the entire puc region was lethal, whereas we found that deletion of the entire pucBACDE region was not lethal or generally deleterious to cell growth.

The deletion analysis described here shows that two of the three genes downstream of pucBA are required to obtain normal levels of the LHII complex. One of these genes, pucE, encodes one of the 14 kDa proteins that copurify with LHII complex α and β pigment-binding proteins (Tichy et al., 1989), and its deletion impaired LHII complex formation. This pucE deletion strain synthesized less LHII complex than the wild-type (see Fig. 3c; in several experiments the values of the areas under the 800 nm peaks were approximately 65% of the wild-type controls for low-oxygen cultures, and about 37% for photosynthetically grown cultures), and the absorption of the 800 nm peak seemed to be disproportionately low relative to that at 850 nm. This 800 nm absorption peak is probably associated with the periplasmic side of the complex (Fowler et al., 1992).

Deletion of the pucC gene leads to a complete loss of LHII complex absorption. Both of our pucC and pucE deletion results are consistent with the work of Tichy et al. (1991). In contrast, we found that deletion of the pucD gene had no apparent effect on LHII complex levels detected by spectroscopy. This difference could be explained by a secondary effect on expression of pucC if the pucD deletion used by Tichy et al. (1991) was not translationally in-frame, and translational coupling between the pucD and pucE genes was therefore lost (see below).

The appearance of secondary mutants that express LHII complexes in low-light-grown photosynthetic cultures of ΔLHII(pAC) and ΔLHII(pACDE) implies that at least one other gene is capable of providing the function of the missing pucC gene, and presumably its own normal function, when modified due to a mutation. We have evidence that these mutations are chromosomally located in both ΔLHII-1(pAC) and ΔLHII-2(pACDE), since transfer of the plasmids from these strains to strain ΔLHII gave the same phenotypes as plasmids pAC and pACDE (unpublished data). The data presented here do not distinguish whether the differences in absorption spectra of ΔLHII-1(pAC) and ΔLHII-2(pACDE) are due to different suppressor mutations, or result from the presence or absence of the pucE genes.

Two candidates as sites for a mutation that suppresses the effects of pucC gene deletion are ORF1696, found upstream of the puhA gene, and ORF428 [located between the bchG and bchJ genes; Burke et al., 1991, sequence obtained from GenBank (accession Z11165)]. The amino acid sequence of ORF428 has 26% identity to PucC, but the function of this putative gene has not yet been evaluated. However, the predicted amino acid sequence of ORF1696 has 47% identity to that of PucC (Bauer et al., 1991), and mutations in ORF1696 have been shown to decrease levels of the LH1 complex by about 30% (Bauer et al., 1991). We therefore speculate that the function of ORF1696 in the assembly of LH1 complexes is analogous to that of pucC in the assembly of LHII complexes, and that mutation of ORF1696
allows it to compensate for the deletion of *pucC*. This hypothesis is under investigation.

**Growth characteristics of *puc* gene deletion strains**

A surprising result of the growth studies of the mutants described in this report was that deletion of the *pucC* or *pucE* genes had a more deleterious effect on photosynthetic growth than deletion of the entire *puc* operon (see Fig. 4). Compared to the photosynthetic growth of strain Δ*LHII*(pBACDE), the strain Δ*LHII*(pRK415::Ω) grew equally well at 300 μmol m⁻² s⁻¹ and only slightly more slowly at 30 μmol m⁻² s⁻¹. In contrast, strains Δ*LHII*(pΔC), Δ*LHII*(pΔCDE) and Δ*LHII*(pΔE) grew very poorly, even though the Δ*LHII*(pΔE) strain had about 37% of the wild-type levels of the LHII complex. It would thus appear that expression of the *pucBA* genes interferes with the photosynthetic growth capabilities of cells in the absence of the *pucC* or *pucE* genes. No growth significant differences were seen in aerobic dark rates (data not shown).

**puc operon promoter location by deletion analysis of *pucE*:::lac'Z gene fusions**

Promoter activity was not detected between the *pucA* and *pucC* genes (Tichy et al., 1989), although transcription initiated at the *nifHDK* promoter could proceed through the inverted repeat sequence located between these two genes to allow expression of *pucC* (Tichy et al., 1991). However, it was not clear if the *pucBA* promoter (as opposed to the *nifHDK* promoter) could drive transcription of the *pucCDE* genes, or whether another promoter was present in the region downstream of the *pucC* gene. Our results, with a fusion between the *pucE* and *lacZ* genes, show that at least 90% of transcription of the *pucE* gene originates in the previously mapped *pucBA* promoter region (Fig. 5), and that this transcription has a similar degree of oxygen regulation to that of *pucB*-lac'Z fusions (Forrest et al., 1989). The remaining 10% of promoter activity associated with the region between the *BsaBI* site in the 3' end of the *pucC* gene and the *HincII* site in the *pucD* gene could indicate the presence of a minor promoter in this region. Alternatively, the low level of transcription in the pCEZ and pBEZ constructs may be due to a low level of read-through from a plasmid promoter. With either possibility, the decrease in activity between the pBEZ and the pHEZ constructs (Fig. 5) could represent a contribution of translational coupling of the *pucD* gene to expression of *pucE*, since these genes overlap by four nucleotides (Tichy et al., 1989). That is, if these genes were translationally coupled, translation of the *pucE* message would decrease when the 5' end of the *pucD* gene (including the ribosome binding site and start codon) was deleted. Although our results show that most of the expression of the *pucE* gene derives from transcription initiated at the *pucBA* promoter, the data do not preclude the possibility of a small contribution by a minor promoter between the *pucC* and *pucD* genes, translational coupling between the *pucD* and *pucE* genes, or some combination of these possibilities.

**RNA blot analysis of the *puc* operon region shows multiple RNA species**

Localization of nearly all of the promoter activity for transcription of the *pucE*:::lac'Z fusion to the region upstream of *pucE* implied that it might be possible to detect an RNA species sufficiently long to encode all five *puc* genes. As shown in Fig. 6b, the 2.2 kb *pucBACDE* probe detected messages of approximately 0.5 kb, 1.0 kb and 2.4 kb. The 0.5 kb signal was the most abundant and was detected by the *pucBA* probe, but not the *pucC* or *pucDE* probes, indicating that it represents the two previously characterized approx. 550 nt *pucBA* mRNAs (Zucconi & Beatty, 1988).

The 1.0 kb RNA species detected with the *pucBACDE* probe was approximately 10-fold less abundant than the *pucBA* mRNA. A strong signal migrating to the same position was detected by a *pucDE*-specific probe, and a signal of the same size was also detected using a probe to the *pucC* region. These results could either be due to two similarly-sized species, or the 3' end of the *pucC* probe could overlap with the 5' end of the RNA molecule detected by the *pucDE* probe. The distance from the 3' end of the *pucC* probe to the presumed transcriptional terminator downstream of *pucE* (Tichy et al., 1989) is approximately 1-2 kb, which is about the size of the RNA species detected by both probes. If these probes did hybridize with the same RNA molecule, this 1.0 kb species would be more likely to have been generated by processing of a primary transcript than by promoter activity, since none was detected in this region by the *pucE*:::lac'Z fusions (see above). The *pucE*:::lac'Z fusions indicated that there might be minor promoter activity in the region between the *BsaBI* site near the 3' end of *pucC* and the *HincII* site within *pucD*. Transcripts originating from this potential minor promoter and ending at the supposed transcriptional terminator downstream of *pucE* (Tichy et al., 1989) would be approximately 0-7 kb, and a minor band of 0.7 kb was detected using the *pucDE* probe. This signal would not be visible using the larger *pucBACDE* probe because it would be masked by the 0.5 kb signal.

All of the probes used detected signals of a RNA species migrating at about 2-4 kb. Combined with the results of the *pucE*:::lac'Z fusions, this strongly implies...
that transcripts encoding all five puc operon genes originate at the pucBA promoter. The presence of smaller species, especially the 1.0 kb species detected by the pucBA probe, and the relatively low abundance of the 2.4 kb message, imply that a 2.4 kb primary transcript is degraded to generate more stable products. It remains to be seen if the 3' end of the primary transcript is degraded to generate more stable species, especially the 1.0 kb species detected by the pucC probe.

Analysis of puc RNA in the related species R. sphaeroides demonstrated the presence of a 2.3 kb message having the same 5' end as a more abundant 0.5 kb pucBA transcript (Lee et al., 1989). The authors concluded that this 2.3 kb molecule was required for formation of the LHII complex. It has not been reported whether or not R. sphaeroides contains pucCDE gene homologues. Although transcripts of the pucBA genes continue into the region downstream of pucA and this downstream region is associated with LHII complex formation in both R. sphaeroides and R. capsulatus, the specific functions of the pucC, pucD and pucE gene products in LHII complex formation remain to be determined.

References


